



2001-1251

PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Maria Anna WUBBEN et al.

Conf. No. 1786

Serial No.: 08/776,321

GROUP: 1761

Filed: April 15, 1997

Examiner: Curtis Sherrer

Title: PECTINS AS FOAM STABILIZERS FOR BEVERAGES

HAVING A FOAM HEAD

RECEIVED
JAN 12 2004
TC 1700

RULE 132 DECLARATION OF A.C.A.P.A. BEKKERS

The undersigned, Augustinus Cornelius Aldegonde Petrus Albert Bekkers, Nijenheim 11-87, 3704 AE Zeist, the Netherlands, herewith declares as follows:

1. I have been an employee of Heineken Technical Services BV, at Zoeterwoude, the Netherlands, the assignee of the present invention, for over four years. I am a graduate of Utrecht University with a specialization in biochemistry, obtained a Ph.D. at Utrecht University in biochemistry and was formerly employed for about five years as a food chemist at TNO Nutrition and Food Research Institute. For about 2 years I have been involved in isolating and functional testing of pectin from hop.
2. In order to analyze the composition of hop extracts as described by Hoelle et al (US 3,222,181), the extraction and reconstitution of hop constituents as described by Hoelle et al was performed under my supervision.
3. The extraction methodology by Hoelle et al is not always described in full detail. In those cases where the teachings of Hoelle et al are not unambiguous, we have chosen the option that we believe will produce the highest yield of pectin and/or will preserve the polymeric nature of the hop pectin. Thus, we chose (i) to use dry hop as a starting material, (ii) to not reuse the organic extract for multiple extractions and (iii) to remove trichloroethylene from the extraction

residue under reduced pressure at ambient temperature. For a detailed description of the method used, see Appendix 1. The method described by Hoelle et al yields an organic extract and an aqueous extract. The recombined extract that is advocated by Hoelle et al for use in beer (claim 2) is an approximately 3:1 mixture of the organic extract and aqueous extract (on a weight basis).

4. Hoelle et al teach (column 3, lines 62-65) that the bulk hops should be comminuted to the extent that hop particles are obtained having a particle size preferably averaging about 50 to 1000 μm . Analysis of the particle size distribution after Ultra Turrax™ milling/extraction of the hops using light scattering techniques showed that indeed this demand on particle size was met (see Appendix 2: "Malvern MasterSizer" analysis).
5. Hoelle et al also teach (column 4, lines 13-18) that by carrying out the comminution of the hops in the presence of solvent in a comminutor device, it is possible to obtain a sufficient increase in surface area of the hops that the extraction is substantially completed within a few minutes. In order to check whether indeed the organic extraction as performed by us, i.e. Extraction (1), was substantially completed, a second extraction, i.e. Extraction (2), was done by re-suspending the spent hops A-1 obtained from Extraction (1) in trichloroethylene.
6. For all extractions, a 10 times volume over weight ratio was used. The extracts were dried under reduced atmospheric pressure at room temperature.
7. In order to make a mass balance, the hop starting materials as well as the extracts and spent hops fractions obtained therefrom were all weighed accurately. In addition, hops, extracts and fractions were analyzed for their uronic acid content, as described in Appendix 3. The quantity of uronic acids found in the samples, which includes anhydrogalacturonic acid, is used as a measure of the maximum possible amount of pectin in the extracts. The uronic acid content of the hops, extracts and spent hop fractions as set forth herein were determined by an independent third party expert: Dr. H. Schols, Wageningen University, Wageningen, the Netherlands.

8. The results obtained were as follows:

Table 1: Mass balance

Sample	Dry weight (g)	Extraction Yield (%)	Uronic acid content (% w/w)	Extraction Yield (%)
Extraction (1)				
dry hop	100.8	(100)	8	(100)
org. extract-1	16.6		0	
spent hops A-1	75.1	Σ 91	10	Σ 94
Extraction (2)				
spent hops A-1	16.6	(100)	10	
org. extract-2	0.0		n.d.	
spent hops A-2	15.6	Σ 94	n.d.	n.d.
Extraction (3)				
spent hops A-1	58.5	(100)	10	(100)
aqueous extract	11.1		3	
spent hops B	45.8	Σ 97	12	Σ 98

Σ = on the basis of sum of extract + spent hops

n.d. = not determined

For an explanation of the terms used in Table 1, see Appendix 1.

9. From the results presented in Table 1 I conclude that an accurate mass balance was obtained for the extraction procedure performed after Hoelle et al. Minor losses of material occurred during filtration as evidenced by the non 100% yields. Like Hoelle et al, we found that a single organic extraction is sufficient to extract essentially all organic solubles. The organic extract obtained is deficient of any uronic acids, indicating that essentially no pectin is present in this extract. The aqueous extract and the spent hops, however, did contain uronic acid, in the amounts indicated.

10. As is evident from Table 1, the aqueous extract obtained according to Hoelle et al. contained 3% uronic acid by dry weight. It is noted that the uronic acid content of 8% w/w of the dry hop starting material is exceptionally high as evidenced by the fact that table 2.1 (page 4) of the European Brewery Convention Manual of Good Practice (1997) indicates that dried hop cones typically contain 2% w/w of pectin and page 5, line 11 of the present application which mentions a range of 1-3% (d.s.). Given that the anhydrogalacturonic acid content of the samples cannot exceed the measured uronic acid content, I conclude that Hoelle et al teach aqueous extracts from spent hop that contain anhydrogalacturonic acid in an amount that is substantially below 20% by weight of dry matter, and, specifically, not more than about 3%. When the aqueous extract is combined with the organic extract in the approximately 1:3 ratio taught by Hoelle et al, the maximum possible anhydrogalacturonic acid content will drop still further. This conclusion remains valid if an organic solvent other than trichloroethylene were used (see column 3, lines 26-35 of Hoelle et al), because the pectin is not extracted in the organic fraction.
11. On the basis of my experience in isolating and analyzing pectin derived from hop, as described above, it is furthermore clear to me that the extraction techniques taught by Hoelle et al are incapable of producing a hop extract containing anywhere near the minimum 20% anhydrogalacturonic acid content required by the pending claims 52-57. This is because, under the aqueous extraction conditions taught by Hoelle et al (100 °C for 20-40 min at pH 8-9; see column 6, lines 29-31 and 38-42), various water soluble components will be extracted in such quantities that pectin will constitute no more than a small fraction of the extracted material. Indeed, this is why in the extraction methods employed in accordance with the present invention the aqueous hop extract is submitted to a precipitation step to selectively isolate pectin from other water soluble components contained within the same aqueous extract (see present application page 6, line 33 to page 7, line 13; ANNEX 1 on page 14; and ANNEX 1 on page 20).
12. I further declare that all statements made herein are true and that all statements made on information and belief are believed to be true; and further that that these statements are made with the knowledge that

willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements and the like so made jeopardize the validity of the document, or application, or any patent issuing thereon.

Signed this Monday 22 day of December 2003

By


Augustinus Cornelius Aldegonde Petrus Albert Bekkers

Appendix 1: Extraction procedure, after Hoelle et al

Extraction (1)

Dry Hops (Hallertau "Magnum", harvest 2003)
↓ Milling using UltraTurrax™
Extraction with trichloroethylene
↓ Filtration
Organic extract-1
+
Spent Hops A

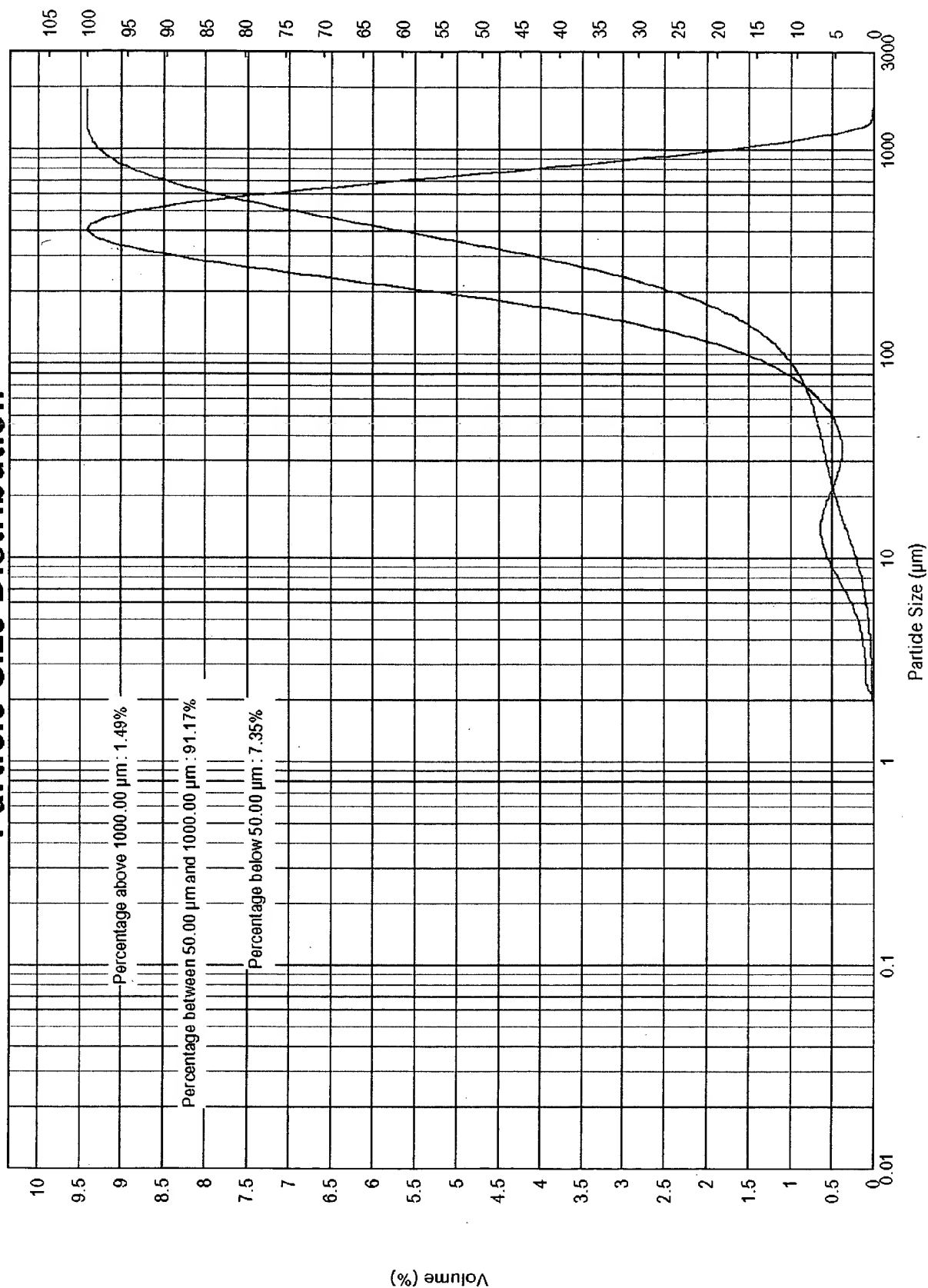
Extraction (2)

Spent hops A-1
↓ Second extraction with trichloroethylene
Filtration
Organic extract-2
+
Spent Hops A-2

Extraction (3)

Spent Hops A-1 Dry
↓ Suspend in water pH 8.5, boil for 20 minutes
Filtration
Watery extract
+
Spent hops B

Particle Size Distribution



— HOELLE 20-10-2003 exp.1 triplo - Average, Monday, October 20, 2003 12:39:32 PM

Makern Instruments Ltd.

Malvern, UK

Tel : +44 (0) 1684-892456 Fax +44 (0) 1684-892789

Masterizer 2000 Ver. 6.1

Serial Number : MAL100591

File name: Malvern Leuven.mea

Record Number: 153

22 Dec 2003 2:55:19 PM

Appendix 3:

Uronic acid content

The uronic acid content was determined as anhydro-uronic acid (AUA) by an automated *m*-hydroxydiphenyl assay [1,2] using an autoanalyser (Skalar Analytical BV, Breda, The Netherlands).

References

- [1] Blumenkrantz, N. & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry*, 54, 484-489.
- [2] Thibault, J. F. (1979). An automated method for the determination of pectic substances. Automatisation du dosage des substances pectiques par la methode au meta-hydroxydiphenyl. *Lebensmittelen Wissenschaft Technologie*, 12(5), 247-251.